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Acute Visceral Toxoplasmosis in Captive Dik-Dik (*Madoqua guentheri smithi*)

J. P. Dubey, Maryanne E. Tocidlowski*, Bruce Abbitt†, and S. Y. Llizo*, Parasite Biology, Epidemiology, and Systematics Laboratory, Animal and Natural Resources Institute, Agriculture Research Service, United States Department of Agriculture, BARC-East, Building 1001, Beltsville, Maryland 20705-2350; *The Houston Zoological Gardens, 1513 N. MacGregor, Houston, Texas 77030-1603; and †Texas Veterinary Medical Diagnostic Laboratory System, P.O. Drawer 3040, College Station, Texas 778431-3040. *e-mail: jdubey@anri.barc.usda.gov*

ABSTRACT: Acute toxoplasmosis was diagnosed in 2 captive dik-dik (*Madoqua guentheri smithi*) in the Houston Zoo. Both animals became ill suddenly and died in spite of supportive therapy. *Toxoplasma gondii* was identified in tissues of both animals immunohistochemically, and antibodies to *T. gondii* were found in titers of 1:800 or more in both animals upon examination by the modified agglutination test. The cause of death was considered to be toxoplasmic pneumonia. This is the first report of toxoplasmosis in *M. g. smithi*.

Infections by the protozoan parasite *Toxoplasma gondii* are widely prevalent in animals including humans throughout the world (Dubey and Beattie, 1988). Among zoo animals, they are a major cause of mortality in Australasian marsupials, e.g., different wallabies, New World monkeys, certain species of birds, e.g., canaries, and certain species of felids, e.g., Pallas cats (Dubey and Odening, 2001). Infection in zoo cats is of public health significance because of the danger of contamination of the zoo environment. Furthermore, felids are the only hosts that can excrete environmentally resistant *T. gondii* oocysts. The objective of the present paper is to document acute fatal toxoplasmosis in dik-dik (*Madoqua guentheri smithi*). This is the first report of *T. gondii* infection in this animal species.

Two animals were affected. Case 1 (16474) was a 17-mo-old female dik-dik born in the Houston zoo on 9 July 1996. While on exhibit, the dik-dik was noted to be lethargic, slightly ataxic, and trembling. The animal was brought to the zoo's hospital for further examination and treatment. It was found to be thin and dehydrated, with slightly labored respiration. Blood was collected and the animal was given supportive therapy. Thoracic radiographs revealed a possible interstitial pneumonia. The animal was given cefiofur sodium (Naxcel®, Upjohn Co., Kalamazoo, Michigan) and B-complex vitamins. The animal became progressively weaker throughout the day and died the next day. A blood sample was obtained on 12 December 1997, a day before death, and necropsy examination was performed the next day.

Case 2 (14185) was a 7-yr-old female, 3.9 kg dik-dik born in the Houston Zoo on 25 January 1993. The animal was hospitalized in July 2000 because of a simple midshaft fracture of the left metacarpal bone, which healed without incident. While hospitalized for convalescence, blood samples were obtained on 11 July, 10 August, and 6 September 2000. The animal was returned to its exhibit on 5 October. In the morning of 14 November, the dik-dik was found moribund at the exhibit and brought to the zoo's hospital for treatment. The animal was severely hypothermic (<32 C) and nonreactive to stimuli. Treatment consisted of warmth, intravenous fluids, steroids, and trimethoprim–sulfadiazine (Tribrissen®, Coopers Animal Health Inc., Kansas City, Kansas). A blood sample was collected for laboratory analysis and serum banking. A free-catch, hemorrhagic urine sample was submitted for analysis. The animal died early in the morning of 15 November, and necropsy examination was performed the same day.

Complete necropsies were performed at the Houston Zoo. Specimens of major organs were fixed in buffered neutral 10% formalin. Paraffin-embedded sections were cut at 5-μm thickness, stained with hematoxylin and eosin, and examined microscopically. Retrospectively, paraffin sections of certain organs were stained immunohistochemically (IHC) with *T. gondii* and *Neospora caninum* rabbit polyclonal antibodies using methods described previously (Lindsay and Dubey, 1989). Certain sections were stained with anti–BAG-1 antibodies to *T. gondii* as described previously (McAllister et al., 1996). This BAG-1 antibody is specific for bradyzoites and does not react with tachyzoites at 1:10,000 dilution. *Toxoplasma gondii* tissue cysts from the brain of a chronically infected mouse and the tissues of a mouse inoculated with RH Strain tachyzoites were included in the IHC tests; the RH Strain does not form tissue cysts in mice. Serum samples were tested for antibodies to *T. gondii* by the modification agglutination test (MAT) as described previously (Dubey and Desmonts, 1987).

Gross lesions were found in both cases. In Case 1, the lungs were edematous, mottled, and bilaterally uniform. There were pale areas in the myocardium, the lymph nodes were enlarged, fat was atrophied, and the small intestine was hemorrhagic. In Case 2, the lungs were edematous and red. The mediastinal and mesenteric lymph nodes were enlarged, and the urinary bladder contained a large blood clot.

Microscopically, the main lesions in both animals were in the intestines and lungs (Figs. 1, 2). In Case 1, the lamina propria of the small and large intestines had multifocal areas of necrosis infiltrated by neutrophils and histiocytes (Fig. 1). Individuals and groups of *T. gondii* tachyzoites were present in the lamina propria (Fig. 1E). The liver had multifocal areas of necrosis and accumulations of lymphocytes, macrophages, and neutrophils. Tachyzoites were seen in hepatocytes, often at the periphery of lesions (Fig. 2E). In the lungs, there was diffuse necrosis of alveolar septal tissue infiltrated by neutrophils, lymphocytes, and macrophages (Fig. 2A). The alveolar lumen contained fibrin and type II pneumonocytes. Protozoal tachyzoites were present. The adrenal gland had multifocal areas of parenchymal necrosis and accumulations of neutrophils, lymphocytes, and marcophages in the cortex and medulla, and protozoal tachyzoites were present in lesions. The brain had multifocal leptomeningitis and scattered focal accumulations of mononuclear cells around small areas of necrosis; protozoans were not seen. The heart had rare foci of necrosis and focal accumulations of mononuclear cells associated with protozoa. The kidneys had focal areas of necrosis and accumulations of mononuclear cells, and protozoans were seen in lesions. The lymph node had edema and histiocytosis; the protozoa were not identified. Protozoans in the heart, lung, liver, adrenal gland, kidney, and intestine reacted positively with anti–*T. gondii* antibodies but not with anti–*N. caninum* antibodies. Protozoans in the heart and adrenal gland reacted positively with anti–BAG-1 antibodies. Lesions or protozoa were not seen in sections of the abomasum, spleen, uterus, skeletal muscle, urinary bladder, and rumen. The MAT antibody titer to *T. gondii* in the serum of this animal a day before death was 1:800.

In Case 2, the most severe lesion was pneumonia. Diffusely, there was expansion of the alveolar septa by accumulations of mononuclear cells, and filling of alveoli by edema, fibrin, and macrophages. Protozoans were present in lesions (Fig. 2A–D). There were focal inflammatory lesions in the rumen and reticulum (Fig. 2A–C), adipose tissue in the mesentery, focal adenitis in the lymph nodes, and focal hemophage in the urinary bladder and large intestine (Fig. 2D).

Protozoans in the lung, liver, spleen, esophagus, rumen, reticulum (Fig. 2A–C), heart, urinary bladder muscle, and large intestine reacted with both polyclonal and anti–BAG-1 sera. Tissue cysts in lungs were stained with periodic acid Schiff reagent (Fig. 2D).

Antibodies to *T. gondii* were not found in a 1:25 dilution of serum samples obtained in December 1997 and the 3 samples obtained in July, August, and September 2000. The antibody titer (MAT) of the serum obtained a day before death was ≥1:3,200.

The diagnosis of toxoplasmosis in the present study was based on immunohistochemical identification of the parasite in tissue sections and the presence of antibodies to *T. gondii* in serum samples. The finding of *T. gondii*–associated lesions in visceral tissues (intestine, liver, lung) indicates that both animals had acquired *T. gondii* infections recently because no parasites were found in their brains. One animal had focal lesions (necrosis and infiltration of macrophages) suggestive of recently acquired infections. Although the source of *T. gondii* infection in the present study was unknown, the character of the lesions suggests recent

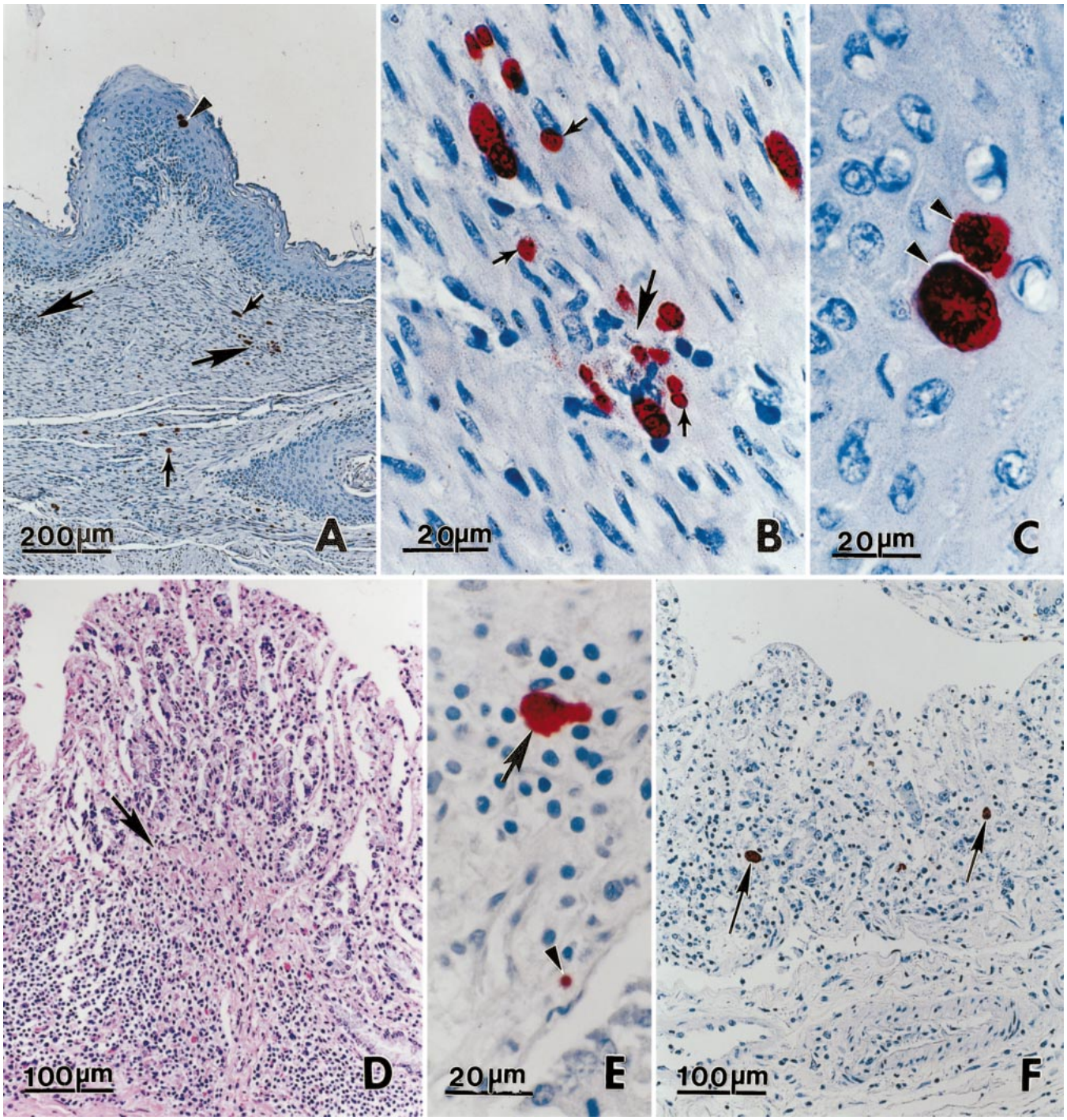

FIGURE 1. Lesions and *Toxoplasma gondii* in the reticulum (A–C) and colon (D–F) of dik-dik 2 (A–C, F) and 1 (D, E). (A–C, E, F) IHC with anti–*T. gondii* polyclonal serum (A–C, E) and anti–BAG-1 serum (F), and hematoxylin and eosin stain (D). (**A**–**C**) Foci of inflammation (large arrows) and *T. gondii* in submucosa (small arrows) and in mucosa (arrowheads). (**D**) Focus of necrosis and inflammation in the submucosa (arrow). (**E**) A group of tachyzoites (arrow) and 1 tachyzoite (arrowhead). (**F**) Two tissue cysts (arrows) in the lamina propria.

ingestion of oocysts. In animals fed large numbers of oocysts, *T. gondii* multiplies in the intestine and associated lymph nodes and then spreads to other organs (Dubey and Beattie, 1988). Some animals can die before lesions develop in the brain. The character of the lesions in the animals in the present study suggests such a scenario. It is of interest that the lesions in both animals were essentially similar although death occurred 3 yr apart. In Case 2, the animal was seronegative in September 2000, 2 mo before it died. Thus, the infection was likely acquired between September and November. Animals usually become seropositive for *T. gondii* by MAT by 3 wk postingestion of oocysts (Dubey and Beattie, 1988).

The finding of *T. gondii* and lesions in the rumen and reticulum is of interest because *T. gondii* is not known to parasitize these organs. The finding of BAG-1 positive organisms in several visceral tissues including the intestine, rumen, and reticulum is also unusual. Reactivity with anti–BAG-1 antibody indicates the presence of bradyzoites be-

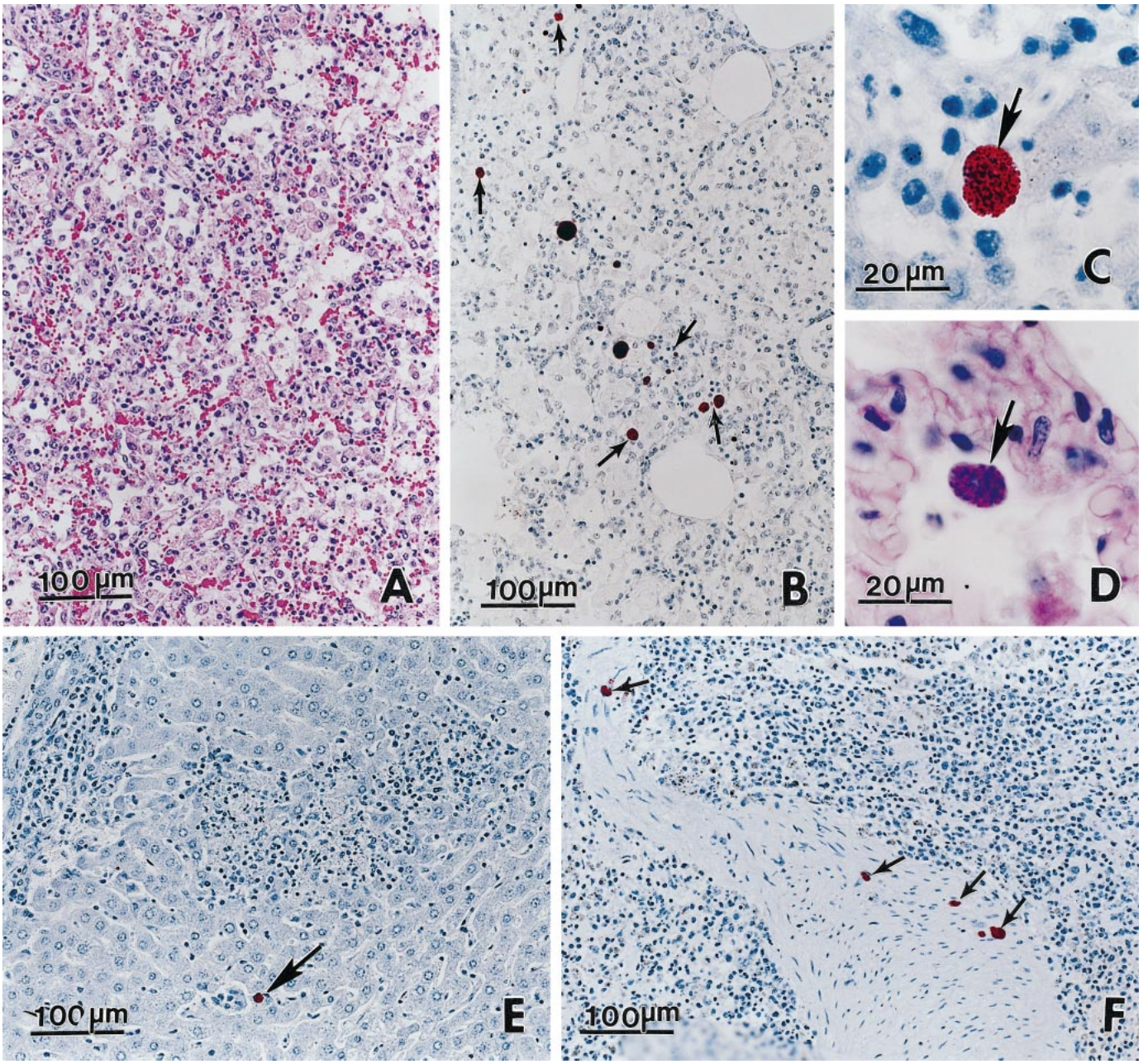

FIGURE 2. Lesions and *Toxoplasma gondii* in tissues of dik-dik 2. (A–D) Lung; (E) liver; and (F) spleen. (**A**) Edema and infiltration of mononuclear cells in alveoli. Hematoxylin and eosin stain. (**B**) Several groups and individual *T. gondii* (arrows). IHC stain with anti–*T. gondii* polyclonal serum. (**C**) Tissue cyst (arrow), IHC stain with anti–*T. gondii* BAG-1 serum. (**D**) Tissue cyst (arrow) in alveolus. Periodic acid Schiff hematoxylin. (**E**) Cholangio-hepatitis with a group of *T. gondii* (arrow) in a hepatocyte, IHC stain with anti–*T. gondii* polyclonal serum. (**F**) Spleen with BAG-1 positive *T. gondii* (arrows) in trabelculae, IHC stain with anti–BAG-1 *T. gondii* serum.

cause this antibody does not react with tachyzoites. *Toxoplasma gondii*–infected mouse tissues were included as controls in the present study and were not stained with the anti–BAG-1 serum.

The dik-dik were housed in an outdoor, high-walled, open exhibit along with a small troop of Patas monkeys (*Erythrocebus patas*). It is possible for feral cats to enter the exhibit and contaminate the food and water. The hay fed to the animals could also have been contaminated by feral cat feces either when stored off grounds at the commercial site or while on zoo grounds.

Dik-diks are African antelopes belonging to the family Bovidae. They are hunted widely in Africa, and their skin is used for the manufacture of gloves. Although there is no report of toxoplasmosis in dik-dik, fatal toxoplasmosis was reported in Saiga antelope (*Saiga tatarica*) by Bulmer (1971) and Ippen et al. (1981). Burgisser (1960) reported fatal toxoplasmosis in 2 unnamed deer. Although fatal toxoplasmosis has not been reported in the pronghorn (*Antilocapra americana*), the pronghorn is highly susceptible to infection (Dubey et al., 1982). Generalized visceral toxoplasmosis was reported in experimentally infected pronghorns (Dubey et al., 1982) and reindeer (Oksanen et al., 1996).

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